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TO ANOXIA OF RABBITS, DOGS AND
GUINEA PIGS FROM THE ONSET OF
VIABILITY TO ADULT LIFE

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

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THE RATE OF DECLINE IN RESISTANCE TO ANOXIA OF RABBITS, DOGS AND GUINEA PIGS FROM THE ONSET OF VIABILITY TO ADULT LIFE^{1,2}

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The present investigation was undertaken to determine how long newborn rabbits, dogs and guinea pigs can survive when breathing nitrogen instead of air. Embryologists have long recognized the fact that when deprived of oxygen, the fetus survived longer than the mother under similar circumstances.

Certain previous investigations are of special interest. Le Gallois (1), in 1813, noted that in newborn rabbits, following decapitation, respiratory movements persisted for 15 minutes; in asphyxia by submersion, 27 minutes; after opening the thorax, 30 minutes; and following extirpation of the heart, 20 minutes. Thirty days after birth, the adult type of response was found and respiration ceased within 2 minutes.

Paul Bert (2) observed in newborn rats the persistence of respiratory movements for 30 minutes during submersion in water. Twenty days after birth, respiration ceased in about $1\frac{1}{2}$ minutes. He also noted that cat litter mates submerged in water at 20 degrees survived 26 minutes, while at 36 degrees the time of survival was only $11\frac{1}{2}$ minutes.

Observations have also been made upon animals placed in a bell jar into which various gases were introduced. By this method, Reiss and Haurowitz (3) and Avery and Johlin (4) showed that newborn mice were more resistant than adults, whether anoxia was produced by carbon monoxide, hydrogen, carbon dioxide or cyanide. The work of Fazekas, Alexander and Himwich (5) further substantiated this fact.

Kabat (6) found that, after interruption of the blood supply of the brain, the time of survival of the newborn dog was four times that of the adult. By ligation

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of vessels supplying the brain, as well as by decapitation, Selle and Witten (7) observed that the duration of gasping movements decreased with age.

The foregoing investigations have been limited to animals at term and during the neonatal period, and do not include observations on the survival time at earlier stages of development. The observations to be reported in this paper deal largely with the resistance to anoxia at various stages of fetal life and center about five main questions:

1. When nitrogen instead of air is breathed, what is the survival time of the normal newborn rabbit compared with that of the adult?
2. Is survival time increased or decreased in premature fetuses?
3. If the newly born survives longer than the adult, what is the duration of the period of transition from the newborn to the adult type of response?
4. Does a 3-day old rabbit living in the nest survive as long as a postmature fetus living in the uterus 3 days past term; or what is the influence of intrauterine environment on survival time?
5. In the comparison of various species (rabbit, guinea pig and dog) what is the relation of the stage of development and length of gestation period to survival time?

METHODS AND MATERIAL. Kymograph records of the respiratory movements were obtained on every animal throughout the experiment. In the smaller, newborn animals a plethysmograph connected with a recording tambour was used, as previously described by Rosenfeld and Snyder (8). The respiratory movements of the thorax and abdomen were readily observed through the transparent glass chamber which surrounded the animal. Rubber sheeting was fastened over the end of the above chamber and the head of the animal projected through a hole in the sheeting, of such size as to fit snugly around the neck without leakage of air and yet not so tight as to obstruct respiration. A second outer chamber of glass through which nitrogen circulated briskly was then placed over the end of the first chamber and the head of the animal. A similar arrangement using rubber sheeting as described above was used to prevent leakage of nitrogen. In adult animals, too large for the plethysmograph, respiration was recorded by fastening a small blood pressure cuff around the thorax.

In every animal a record of normal respiration was first obtained. Following this the chamber through which 99.9 per cent nitrogen was being circulated was slipped over the end of the plethysmograph and over the head of the animal. The time of survival was measured from the onset of nitrogen administration until the cessation of respiration as recorded on the kymograph tracing. Observations were made throughout at room temperature, about 70°F.

In fetuses at 29 to 35 days which were delivered by hysterotomy, observations on the litters were begun promptly following birth in order to detect any changes which may occur at this time.

Observations were based upon 174 rabbits obtained from 64 litters at various stages of development ranging from 29 days after conception to adults; 50 guinea pigs obtained from 22 litters; and 28 dogs from 6 litters.

The rabbits were reared in the laboratory and developmental stages were

known with accuracy since ovulation regularly occurs 10 hours after mating. Furthermore, since labor can be inhibited by injecting extract of pregnancy urine a week before term, it is possible to obtain fetuses of extreme postmature development, which have survived in the intrauterine environment 3 days past term (9).

OBSERVATIONS. 1. *Rabbit.* A. *Survival time of rabbits in nitrogen from prematurity to adult stage.* From the beginning it was evident that rabbits born at full term, 31 to 33 days, possessed a striking tolerance to anoxia. When nitrogen was breathed instead of air, at 31 days, respiratory movements continued 34 minutes as an average in 18 newborn animals obtained from 4 litters. At 32 days breathing continued 31 minutes in 14 animals obtained from 3 litters; and at 33 days, the time of survival averaged 27 minutes in 12 animals obtained from 4 litters. In sharp contrast to these, 6 adult rabbits survived only $1\frac{1}{2}$ minutes in nitrogen. Further observations showed that the adult type of response was reached 18 days after birth or about the end of the suckling period. At intermediate stages of development, the time of survival in nitrogen gradually diminished as the age increased. In table 1 are summarized the results from a series of 174 rabbits obtained from 64 litters.

Interesting light is thrown upon the oxygen requirement of fetuses long before birth by observations on fetuses delivered by hysterotomy during the different stages of pregnancy. Resistance to anoxia observed at the onset of viability at 29 days averaged about 30 times as great as in adult rabbits. Thus, in 16 fetuses obtained from 5 litters at 29 days, the time of survival was 44 minutes; in 9 fetuses obtained from 3 litters at 30 days, breathing continued for an average of 41 minutes.

The remarkable tolerance of premature rabbits to anoxia was further borne out by the fact that some were reared to adult life after more than 30 minutes in nitrogen. In these experiments, fetuses were removed from the nitrogen chamber before the final phase of increase in respiratory rate occurred, although a half hour or more had elapsed. Respiratory movements were resumed and, in some cases, a normal development to adult life was noted.

During the course of administration of nitrogen, samples of arterial blood were taken to determine the oxygen and carbon dioxide content, using the method of Van Slyke and Neill (10). Blood was obtained from the carotid arteries, or in certain instances by left ventricular puncture. After two minutes of nitrogen inhalation there was extreme anoxemia, the oxygen content being less than 3 volumes per cent. The carbon dioxide content determined at various intervals during the experiments in different animals was 30 to 60 volumes per cent.

B. *The time of survival of postmature rabbit fetuses.* By the experimental prolongation of pregnancy, fetuses were obtained which had survived in the intrauterine environment for 35 days, or 3 days past term. The question arises whether or not the survival time was altered by the prolonged period of three days within the uterus in contrast to fetuses which had lived in the nest for three days following birth at the normal 32 day term.

Observations revealed no increase in time of survival in nitrogen of animals

living in the intrauterine environment rather than outside of it. In rabbits which had lived in the nest for 3 days following birth at 32 days, the time of survival averaged 17 minutes in 13 animals obtained from 5 litters (table 1). In 6 animals of a litter obtained by hysterotomy at 35 days following experimental prolongation of pregnancy it was found that the time of survival averaged 16 minutes. In addition, in 7 animals obtained from 4 litters following spontaneous prolongation of gestation, the time of survival averaged 17 minutes (table 2). There was evidence that the tolerance to anoxia was related to the stage of devel-

TABLE 1

Survival time of rabbits of various ages

AGE	NO. OF LITTERS	NO. OF ANIMALS	AV. SURVIVAL TIME IN N ₂	AV. WT.
<i>days</i>			<i>min.</i>	<i>grams</i>
29	5	16	44	32
30	3	9	41	38
31	4	18	34	42
32 (term)	3	14	31	47
1	4	12	27	52
2	4	12	20	64
3	5	13	17	53
4	2	4	13	79
5	4	10	11	85
6	4	12	13	90
7	2	6	9	101
8	2	6	7	125
9	2	4	9	111
10	2	4	7	160
11	2	4	5	147
12	1	3	3	128
13	1	2	2.5	230
14	2	5	4	190
15	1	4	2.5	179
16	2	3	2.5	193
17	2	4	2.5	196
19	1	3	1.5	212
Adult	6	6	1.5	696

TABLE 2

Survival time of operatively and spontaneously delivered rabbits

AGE	NO. OF LITTERS	NO. OF ANIMALS	AV. SURVIVAL TIME IN N ₂
<i>Operative delivery</i>			
<i>days</i>			<i>min.</i>
29	5	16	44
30	2	6	41
31 (term)	4	18	31
33	2	7	27
34	2	6	22
35	1	6	16
<i>Spontaneous delivery</i>			
30	1	3	39
32 (term)	3	14	31
33	2	5	26
34	3	9	20
35	4	7	17

opment of the fetus rather than to the environment, whether within the uterus or outside of it.

Observations were also made at various stages of development of animals born normally in contrast to those obtained by hysterotomy. As shown in table 2 the type of delivery, whether operative or spontaneous, had no definite influence upon the time of survival in nitrogen at the various developmental stages studied.

2. *Guinea pig.* Observations on guinea pigs revealed striking differences in the findings with rabbits. The time of survival in nitrogen averaged $4\frac{1}{2}$ minutes in 11 animals of 4 litters examined within 12 hours following spontaneous birth.

In fetuses obtained before labor by hysterotomy near term, the survival time averaged 6 minutes in 9 animals of 3 litters (table 3).

The period of transition to the adult type of response is also markedly shortened. At 7 days following birth the time of survival in nitrogen reached the adult level, namely, 3 minutes. It may be noted, however, that the survival time of the adult guinea pig was twice that of the adult rabbit.

3. *Dog.* The resistance of newborn dogs to anoxia was found to be of the same magnitude as that observed in rabbits at birth, although the duration of intrauterine life of the dog is twice that of the rabbit (table 4). A gradual decrease in survival time occurs until the adult type of response is reached during the fourth week following birth. Adult dogs survived in nitrogen for 3 minutes, or twice as long as adult rabbits.

TABLE 3

Survival time of guinea pigs at various stages of development

AGE	NO. OF LITTERS	NO. OF ANIMALS	AV. SURVIVAL TIME IN N ₂	AV. WT.
<i>days</i>			<i>min.</i>	<i>grams</i>
Term	3	9	6	66
0.5	4	11	4.5	88
1.0	4	6	4.5	83
1.5	2	3	4	109
2	1	3	4	94
3	1	3	4	99
4	3	4	3.5	100
5	2	4	3.5	123
6	3	4	3.5	155
7	3	3	3	170
8	3	3	3	185
Adult		5	3	952

TABLE 4

Survival time of dogs of various ages

AGE	NO. OF LITTERS	NO. OF ANIMALS	AV. SURVIVAL TIME IN N ₂	AV. WT.
<i>days</i>			<i>min.</i>	<i>grams</i>
0.5	2	3	31	223
1.5	2	5	25	210
4	2	4	17	347
7	2	3	14	285
14	3	5	8	614
21	1	2	5	900
28	1	3	3	1225
Adult		2	3	9000

DISCUSSION. The foregoing observations provide abundant evidence of a remarkable defense against asphyxia in the fetus. In all species examined it is evident that at birth there is a special capacity for maintenance of life in the mammalian organism under anaerobic conditions. This is especially striking in species such as the rabbit and dog, which are born in a primitive state of development. Evidence that resistance to extreme oxygen want decreases as structural organization becomes more highly differentiated was brought to light with clarity in rabbits in which observations were carried back to the earliest stages of viability. It was noted that the greatest tolerance to oxygen deprivation in the present series was shown by embryos at the stage when survival outside the intrauterine environment first became possible. Additional evidence in support of the finding that ability to resist anoxia is related to the stage of development rather than external environmental factors was obtained in postmature fetuses which had

been retained within the uterus until the extreme limit of intrauterine existence had been attained. Throughout the period of experimentally-prolonged intrauterine existence there was a steady loss of resistance to anoxia parallel to that which occurred in the external environment of animals at identical stages of development.

The present experiments demonstrate apparently for the first time the magnitude of the changes in resistance to anoxia which occur at various stages of intrauterine life from the onset of viability to extreme postmaturity.

In the neonatal period, the differences in the time of survival in nitrogen of the three species under consideration may be clarified by taking into account the differences in structural differentiation of the dog, rabbit and guinea pig at the time of birth. Although the gestation period of the dog is twice that of the rabbit, and the same as that of the guinea pig, the guinea pig is the only one of the three species which is sufficiently well developed at birth to carry on an independent existence. The newborn guinea pig shows advanced muscular co-ordination, running about the cage and devouring solid food; the eyelids are open and the development of the integument is marked by a conspicuous coat of long hair. In contrast, in the dog and rabbit at birth, muscular co-ordination is deficient, locomotion is limited, nutrition is dependent upon suckling, the eyelids are closed, and integumentary development is characterized by scant growth of hair. It is evident that a clue to the similarity in resistance to anoxia between the rabbit and dog (both species surviving one-half hour in nitrogen at birth in contrast to six minutes in the case of the guinea pig) is found in the close resemblance of these two species in respect to the stage of development at birth despite a marked difference in the length of intrauterine life.

The fact that fetuses possess a defense against anoxia—which in dogs and rabbits at birth amounts to ten times or more that of the adult—throws light upon the metabolic processes by which the energy requirements for rapid growth during embryonic existence are met. While still within the uterus it may be readily demonstrated that the fetus responds immediately to decrease in the oxygen supply by marked alteration in activity (11); the breathing of 4 per cent oxygen is marked by apnea of the fetus while the mother shows marked hyperpnea. The present experiments show clearly that there are available to the fetus resources for the maintenance of respiratory movements which in the adult are absent or inconspicuous.

The increased tolerance of the fetus to anoxia is of special significance in relation to the hazards of the latter part of intrauterine life including the period of expulsion from the uterus. Fluctuation in the degree of oxygenation of fetal blood as a result of increased and prolonged uterine contractions has been observed experimentally in the clinic. In so far as the magnitude and duration of uterine contractions approach the extreme state of tetanic contraction, the resources of the fetus for resistance to anoxia during labor are of obvious importance.

The hazards of parturition come to light in a species like the rabbit in which the umbilical cord is short and the length of the birth canal is many times that

of the fetal body. Despite the defense against anoxia by rabbit fetuses, the fetal mortality was observed to average about 12 per cent (12). Evidence that death was related to asphyxia was supported by findings at autopsy which indicated that the fetuses were alive at the onset of labor.

SUMMARY

The duration of respiratory movements when nitrogen was breathed instead of air was determined by the aid of individual kymograph records of respiration in 174 rabbits, 28 dogs, and 50 guinea pigs at various stages of development from the onset of viability to maturity. Analyses of arterial blood samples showed rapid development of extreme anoxemia throughout these experiments.

When placed in nitrogen immediately following birth at term, breathing continued in rabbits for 31 minutes, in dogs for 31 minutes, and in guinea pigs for 6 minutes.

In rabbits delivered operatively at the onset of viability (29 days), breathing persisted 44 minutes, and in rabbits retained within the uterus 3 days past term (35 days), breathing continued 17 minutes, which corresponded to the time of survival of rabbits suckled 3 days following birth at term. Thus, tolerance to anoxia is related to the stage of development rather than to the environment.

Furthermore, guinea pigs at birth were much less tolerant to anoxia than dogs. Although length of gestation is the same in both, the greater maturity of guinea pigs at birth is well known.

In the suckling period, survival of rabbits at 1 week was 10 minutes; at 2 weeks, 4 minutes; and at 3 weeks, $1\frac{1}{2}$ minutes which is the same as that of the maternal animal. Changes in tolerance to anoxia by the dog during the suckling period parallel closely those of the rabbit.

The defense of the fetus against asphyxia is of significance in face of the increased hazard of respiratory failure during the terminal phase of intrauterine life and the early neonatal period.

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